Learning Objectives

After attending this presentation, participants will be able to:

- Compare and contrast the resistance potential of HCV infection and HIV infection
- Describe the available assays to screen for RAVs
- Describe the prevalence and impact of resistance-associated variants (RAVs) on treatment regimens for hepatitis C virus (HCV) infection
What is a RAV?
Resistance Associated Variant

- Amino acid changes which result in a drug being less active (resistance)
- Viral protein specific: NS3, NS5A, NS5B
- Denoted: (consensus) Y93H (variant)

- Resistance is quantified based on drug activity in vitro
  - How much more drug do you need to get the same antiviral effect?
  - Fold change in Effective Concentration 50% (EC50; 2x, 10x, 100x)
  - Must be correlated with clinical outcomes

Baseline RAVs- occur without prior drug exposure
- Polymorphisms (natural variations) in the viral nucleotide sequence
  - Some happen to result in a change in the amino acid at key positions for DAAs

Key HCV Resistance Concepts

1. HCV resistance associated variants (RAVs) can be present without drug exposure
2. HCV RAVs impact treatment response in specific situations
3. HCV resistance is NOT absolute
4. Patient characteristics are just as important as RAVs (if not more than)
5. Future regimens may obviate the need for most resistance testing

HCV and HIV replication dynamics
Virus characteristics and resistance potential

**HCV**
- $10^{9}$ virions/day
- Error prone viral polymerase ($\times 2$)
- No overlapping reading frames
- Moderate infected cell turnover
- Dynamic replication unit
- Cytoplasmic replication ($t_1/2 \sim 10$ hrs)
- No latent reservoir
- Cure possible

**HIV**
- $10^{9}$ virions/day
- Error prone viral polymerase
- Overlapping reading frames
- Rapid infected cell turnover ($t_1/2 \sim 24$ hrs)
- Static replication unit
- Integrated proviral DNA
- Latently infected T cells
- Control, not cure

HIV-1 vs. HCV Genetic Diversity

<table>
<thead>
<tr>
<th>HCV (9213 sites)</th>
<th>H (9315 sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3 (10%)</td>
<td></td>
</tr>
<tr>
<td>HIV-1 group M</td>
<td></td>
</tr>
</tbody>
</table>

Modeling the HCV resistance barrier

- Pre-existence of resistant variant explains their rapid emergence
- Not all variants will be “fit” enough to persist unless there is drug selective pressure
Targets also impact resistance potential
Protease structure: HCV and HIV contrast

- HCV: shallow and relatively featureless
  - Limited options for optimizing inhibitor binding
- HIV: pocket within the homo-dimer

Protease structure: HCV and HIV contrast

Baseline minority variants impact HIV ART responses

HIV NNRTI data:
- HR 2.5 (1.17-5.36) for VF with minority NNRTI resistance
  - SIMEN BB. JID 2009
- ACTG398

Baseline minority variants have a minimal impact on HCV treatment responses

EBR RAVs
No RAVS: 414/438 (95%) 5%
EBR RAVs: 396/439 (90%) 10%

Population NGS @1%

LDV/SOF
EBR/GZR: TN GT1a
Clinical HCV Resistance

For which regimen/circumstance is there a label recommendation to perform NS5A RAV testing?

1. All GT1a infections prior to PRID
2. All GT1a infections prior to EBR/GZR
3. GT1 treatment experienced patients with cirrhosis prior to LDV/SOF
4. GT1 treatment experienced patients with cirrhosis prior to SOF/VEL
5. RAV testing is not recommended in any label.

Resistance Characteristics of HCV Antiviral Classes

<table>
<thead>
<tr>
<th>Class</th>
<th>Antiviral Potency</th>
<th>Genotype Activity</th>
<th>Resistance Barrier</th>
<th>FDA Approvals</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS3 Protease</td>
<td>+++ to ++++</td>
<td>1, 4 [2, 3, 6]</td>
<td>Low to Moderate</td>
<td>Simeprevir (2013)</td>
</tr>
<tr>
<td>N3B Nucleotide</td>
<td>+++</td>
<td>1-6</td>
<td>Very High</td>
<td>Sofosbuvir (2013)</td>
</tr>
<tr>
<td>NS5B Nucleoside</td>
<td>++</td>
<td>1</td>
<td>Low</td>
<td>Dasabuvir (2014)</td>
</tr>
<tr>
<td>NS5A Inhibitors</td>
<td>+++</td>
<td>1, 4, 6 [2, 3]</td>
<td>Low to Moderate</td>
<td>Ledipasvir (2014)</td>
</tr>
</tbody>
</table>


Why NS3 PI resistance is not a big deal

1. Baseline RAVs are not a significant clinical issue
   • RAVs at key PI positions are rare (<1%) without drug exposure
   • No impact of Q80K with SOF + SMV at recommended durations
2. After failure selected PI RAVs are “lost” rather quickly
   • Do they still have an impact, even if no longer detectable?
3. Non-PI options are available for sequencing of treatment
   • Eliminates the issue of a persistent effect

The saving grace with PI resistance?

91% of nonSVR with resistance
1a: R155K +/- Q80K
1b: D168V

Real World Data: Impact of prior PI therapy?

• PI failure= PEG/RBV + PI
• Resistance testing results not available
  • Majority did not have baseline testing
• Prior PI failure was associated with a decreased SVR rate
  • OR: 0.4 (0.2-0.9)
Overview of NSSA RAVs

- NSSA RAVs are the class with the most clinical significance
  - GT 1a
  - GT 3
- Present in about 10-15% of patients without prior exposure
  - More prevalent in GT 1b but of limited clinical significance
- The majority of time an NSSA inhibitor will still be used in a patient with NSSA RAVs
- Treatment extension and addition of RBV are key approaches to management of RAVs with currently available therapies

A Word on NSSA Resistance Terminology

The nomenclature of baseline NSSA resistance varies widely in the literature:

- RAPs—resistance associated polymorphisms
  - ANY nonconsensus amino acid at a site associated with resistance to ANY NSSA inhibitor
- Class RAVs—resistance associated variants
  - Specific amino acid substitutions associated with resistance to ANY NSSA inhibitor
- Drug-specific RAVs
  - Specific amino acid substitutions associated with resistance to a particular NSSA inhibitor
  - Different fold-change cut-offs have been used (2x, 5x, 10x, etc)

Baseline NSSA RAVs: A Moving Target

- GT 1a
- GT 1b
Baseline versus selected RAVs

Baseline
- Single variants
- Variable fold change
- Variable prevalence in viral population
- Any patient

Selected
- Multiple variants
- High fold change
- High prevalence in viral population
- "Difficult to treat" populations

Rate of selection of NS5A resistance upon virologic failure
- Varies by regimen and duration
  - PI based
    - Velprevir + telaprevir + LDV: >99%
    - GZR/EBR: 85%
    - EVR: 68%
  - Nucleotide based
    - SOF/LDV: 75%
    - 8 weeks: 65%
    - SOF/VEL: 93% (14/15; majority GT3 and with baseline RAVs)
  - Nuc-based triple
    - SOF/SBV+MOS (≤ 6 weeks): 0% (n=15)
    - SOF + GZR/EBR (≤ 8 weeks): 37% (n=30)

Available Resistance Testing (US)
- Ultra-deep (or next-generation sequencing [NGS]) vs population (Sanger) sequencing
- What is broadly available:
  1. HCV NS5A drug resistance assay
     - NGS with 30% detection level reported
  2. Hepatitis C viral RNA genotype 1/3 NS3/NS5 drug resistance assays
     - RT-PCR with DNA sequencing
- Both assays now available for GT1 and GT3 HCV
  - GT1 assays are subtype specific

**Examples: NS5A resistance genotyping**

**Durability of Treatment-Emergent NS5A RAVs**
- Study assessing NS5A RAVS in pts failing LDV-containing regimens (non-SOF)

**Broad Cross-Resistance With “Early Generation” NS5As**

<table>
<thead>
<tr>
<th>Fold Change</th>
<th>Genotype 1a</th>
<th>Genotype 1b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M28T</td>
<td>Q30R</td>
</tr>
<tr>
<td>Ledipasvir</td>
<td>20x</td>
<td>&gt; 100x</td>
</tr>
<tr>
<td>Ombitasvir</td>
<td>&gt; 500x</td>
<td>&gt; 100x</td>
</tr>
<tr>
<td>Daclatasvir</td>
<td>&gt; 500x</td>
<td>&gt; 100x</td>
</tr>
<tr>
<td>Elbasvir</td>
<td>20x</td>
<td>&gt; 10x</td>
</tr>
<tr>
<td>Velpatasvir</td>
<td>&lt; 10x</td>
<td>&lt; 3x</td>
</tr>
<tr>
<td>ABT-493</td>
<td>&lt; 10x</td>
<td>&lt; 10x</td>
</tr>
</tbody>
</table>
Impact of Baseline NS5A RAVs LDV/SOF

SVR12 by LDV RAV* status with guideline-recommended SOF/LDV treatment


SVR12 (%) by LDV RAV* status with guideline-recommended SOF/LDV treatment

Naïve

Experience

Impact of Baseline NS5A RAVs in Pts With GT1a HCV Treated With EBR/GZR

Analysis of pts with GT1a HCV treated with GZP/EBR in phase II/III trials (naive/relapsers)


GZR/EBR Efficacy in GT1a HCV: Resistance is all that matters!

Analysis of PEP* of TN pts with GT1a HCV treated with GZP/EBR in phase II/III trials

*PEP = pooled efficacy population.

RAVs in DAA experienced patients

Baseline NS5A RAVs Are Associated With Retreatment Failure
Impact of Multiple Negative Predictors on Response

- Retrospective analysis of phase 2/3 studies of SOF + RBV ± PegIFN
- > 850 pts, genotypes 1, 2, and 3 HCV

<table>
<thead>
<tr>
<th>Number of Negative Predictors</th>
<th>SVR12 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>94</td>
</tr>
<tr>
<td>3</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>


What Role Does RBV Play in Overcoming Resistance and Optimizing Retreatment?

- Male: 78%
- 1a: 78%
- HCV RNA: 6.4 (± 0.8)
- Black: 100%
- Non-CC: 100%
- Cirrhosis: 22%
- NSSA RAVs: 78%

SOF/LDV + RBV

EOT SVR12

SVR12 (%)

- Male: 78%
- 1a: 78%
- HCV RNA: 6.4 (± 0.8)
- Black: 100%
- Non-CC: 100%
- Cirrhosis: 22%
- NSSA RAVs: 78%

Genotype 3 and RAVs
In which scenario do the AASLD/IDSA guidelines NOT recommend RAV testing for GT3?

- 1. Treatment experienced patients prior to SOF/VEL
- 2. Treatment naïve cirrhotic patients prior to SOF/VEL
- 3. Treatment experienced and cirrhotic patients prior to SOF/VEL
- 4. RAV testing is never recommended with SOF/VEL

SOF/VEL: Answer for GT3 Cirrhotics and those with Y93H?

NS5A RAVs and Responses to SOF/VEL
When should you do RAV testing?

<table>
<thead>
<tr>
<th>Definitely</th>
<th>Probably</th>
<th>Maybe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Who: All GT1a prior to EBR/GZR</td>
<td>Who: All GT1 DAA failure</td>
<td>Who: GT1a treatment experienced</td>
</tr>
<tr>
<td>What: NS5A (EBR RAVs)</td>
<td>What: NS5 and NS5A</td>
<td>GT3 non-cirrhotic (SOF + DCV)</td>
</tr>
<tr>
<td>M28, Q30, L31, and Y93</td>
<td>5-10% impacted</td>
<td>GT3 TE or cirrhosis (SOF/VEL)</td>
</tr>
<tr>
<td>Action: 1. Extend to 16 weeks</td>
<td>Action: 1. Select non-cross resistant therapy</td>
<td>Action: 1. GT1a-consider RBV with LDV/SOF</td>
</tr>
<tr>
<td>2. Add RBV OR</td>
<td>2. Add RBV (regardless)</td>
<td>• 24wks + RBV with F4</td>
</tr>
<tr>
<td>3. Consider other therapy</td>
<td>3. Extend therapy</td>
<td>2. GT3- add RBV to SOF+DCV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. GT3 TE or cirrhosis- add RBV to SOF/VEL</td>
</tr>
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Drug Resistance-Associated Variants in Hepatitis C Virus Infection: Hype or Help?

David L. Wyles, MD
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